Retinal Vessel Phenotype in Patients with Non-Arteritic Anterior Ischemic Optic Neuropathy.

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

ABSTRACT

Purpose: The pathophysiology of non-arteritic AION (N-AION) is not completely understood. Studies of the retinal vasculature phenotype in patients with N-AION could help us to understand vascular abnormalities associated with the disease.

Design: Retrospective case series with matched controls.

Methods:

Study population: 57 patients with N-AION and 57 controls matched to N-AION patients for sex, age, systemic hypertension, diabetes and obstructive sleep apnea syndrome, between September 2007 and July 2017.

Main outcomes measures: All patients and control subjects underwent a complete ocular examination and 45-degree funduscopic color photographs. The widths of the six largest arteries in zone B (between 0.5 and 1 optic disc diameter from the optic disc), summarized by the central retinal artery equivalent (CRAE), the widths of the six largest veins in zone B, summarized by the central retinal vein equivalent (CRVE), the arteriole-tovenule ratio (AVR), tortuosity and fractal dimension (FD) were measured on the two groups using VAMPIRE (Vessel Assessment and Measurement Platform for Images of the Retina), a software tool for efficient semi-automatic quantification of the retinal vasculature morphology in fundus camera images. Univariate analysis using the Student t-test and MacNemar Chi-squared test for paired sample and Generalized Estimated Equations for modeling the VAMPIRE parameters as dependent variables were used.
Results: CRVE and FD (D0a) were significantly higher in the N-AION group when compared with the control group, whereas the AVR and vascular tortuosity were significantly lower. In comparison with controls, acute N-AION yielded increased CRAE value (174 ±33 vs. 160 ±13 µm) while resolution N-AION yielded decreased CRAE value (152 ±12 vs. 156 ±33 µm). Acute N-AION yielded increased CRVE value (244 ±35 vs. 210 ±21 µm) while resolution N-AION yielded unchanged CRVE value. We found no difference between groups for age, refraction, optic disc diameter, CRAE, and FD.

Conclusions: Retinal vascular parameters were different in our sample between N-AION and control patients, especially at the acute stage of the disease. Our results suggest a normalization of the same parameters at the resolution stage.

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Re: AJO-18-1019, "Retinal Vessel Phenotype in Patients with Non-Arteritic Anterior Ischemic Optic Neuropathy."

Grenoble, 04/03/2019

Dear Editor,

We appreciate your decision to consider a second revision of our manuscript entitled
" Retinal Vessel Phenotype in Patients with Non-Arteritic Anterior Ischemic Optic Neuropathy" which was submitted to the American Journal of Ophthalmology.

We are also grateful to the reviewers for their thorough examination of our manuscript and for the practical presentation of their comments. Please find below item-by-item responses to the reviewers' comments, which are included verbatim for their convenience. All page and paragraph numbers refer to the *revised* manuscript.

All authors have seen and approved the new manuscript. The manuscript has not been published and is not being considered for publication elsewhere.

We hope that the new version of our paper, which has been rewritten taking the reviewers' comments into account, is deemed worthy of publication.

Sincerely yours,

Prof. Christophe CHIQUET, MD, PhD

1. The authors have appropriately acknowledged in the results that the "significant" differences in the D0a values were driven by changes in the control subgroups and likely due to confounding or random variation. [The specific data points are: D0a NAION Acute 1.55 (IQR 1.51-1.59), Resolution 1.55 (1.54-1.59) versus Controls Acute 1.54 (1.48-1.5 and Resolution 1.50 (1.46-1.56)] The statistical association is artifactual and weak enough that likely would not survive correction for multiple comparisons. Despite the acknowledgment in the results, changes in D0a are still claimed in the abstract and the discussion as a major finding of the manuscript. This is not accurate and cannot be allowed. Moreover, the numbers reported in the abstract are not consistent with those reported in Table2. Any reference to change in FD/D0a needs to be removed from the abstract. In the discussion, the reference to D0a needs to be removed from the first paragraph and the entire 5th paragraph needs to be removed. (The first sentence of this paragraph, "We found that fractal dimension (D0a) increased at the resolution stage", is completely false; D0a was 1.55 in the acute phase and 1.55 in the resolution phase.) The only statement in the discussion about FD that I believe is worthy of retaining is at the end of the 7th paragraph where you state that "the stability and repeatability of FD measures is the object of ongoing analysis." Any additional conclusions from this measure are problematic.

- **Data have been corrected accordingly in the abstract**
- **Results concerning fractal dimension are discussed, the 5th paragraph of the discussion has been changed accordingly**

2. The first review requested that the figure include representative images illustrating the morphological differences between vessels in a control fundus and a NAION fundus. This has not been done.

Probably we did not understand exactly what the reviewer asked for. Therefore we added figures with an illustration of fundus images for the measurement of CRAE and CRVE in zone B and FD in zone C, in a control subjects and in a NAION patients. Indeed it is impossible to illustrate an higher CRVE and lower AVR and vascular tortuosity form one image since these measurements are slight and not necessarily visible on one fundus image.

3. The demographics are still not complete. The first review specifically requested that demographics be reported individually for both control subgroups. This is important because the D0a measure suggests the presence of a confounder between these sub-groups.

Table 1 reports age, gender, and main risk factor for NAION such as OSA, diabetes and systemic hypertension. As we stated in the first review, we did not report smoking rates and hypercholesterolemia for our patients and controls. Furthermore, We did not evaluate the body mass index, drugs, or other ocular data such as the size of the optic nerve, intraocular pressure, axial length. This is now discussed as a possible limitation.

4. Please change N-AION to NAION and A-AION to AAION in all instances.
This has been changed accordingly.

Retinal Vessel Phenotype in Patients with Non-Arteritic Anterior Ischemic Optic Neuropathy.

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Short title: Retinal Vessel phenotype in NAION

INTRODUCTION

Anterior ischemic optic neuropathy (AION) is the most common acute optic neuropathy in people over the age of 50, with an incidence of 2–10 per 100,000 people per year.[1] AION results from ischemic events within the optic nerve supplied by the short posterior ciliary arteries. Such events may be related to systemic vasculitis affecting large and medium-caliber vessels in giant cell arteritis (arteritic AION); or to blood flow disturbances in non-arteritic ischemic optic neuropathy (NAION). The main risk factors of NAION are crowded disc,[1] systemic hypertension, diabetes mellitus, and obstructive sleep apnea (OSA).[1–3]

The retinal vasculature provides a unique window to assess noninvasively and directly vasculature segments *in vivo*, and reflects the efficiency of the circulation and the distribution of shear stress. A previous pilot study grading retinal vasoconstriction as absent, mild, moderate or severe [4] reported proximal retinal arterial constriction in 68% of patients with NAION. Retinal vascular impairment may be also due to comorbidities associated with NAION or to the vascular dysfunction of the posterior ciliary arteries. Given that the pathophysiology of NAION is not clearly understood, investigations of the ocular vascular beds may help us to formulate new hypothesis.

Over the last decade, several population-based studies have reported associations of summative measurements of the width of retinal vessels (e.g. AVR), as well as tortuosity and fractal dimension (FD), with a wide range of subclinical (e.g. atherosclerosis, inflammation and endothelial dysfunction) and clinical cardiovascular diseases (hypertension, diabetes mellitus, stroke, kidney and heart diseases).[5,6]

The objective of this study was to characterize the morphology of retinal vessels using well-established retinal vascular parameters, namely the central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), AVR, tortuosity and FD, in eyes with NAION compared to controls matched for major risk factors associated with NAION such as age, diabetes, systemic hypertension, and OSA. We used the VAMPIRE (Vessel Assessment and Measurement Platform for Images of the REtina, Universities of Edinburgh and Dundee, UK) software to collect quantitative measurements of the vascular morphology.[7]

Material and methods

Participants

This study, conducted at the Grenoble-Alpes University Hospital, retrospectively included patients imaged between September 2007 and July 2017. It was conducted in accordance with the ethical principles of the Declaration of Helsinki on medical research in patients and approved by the regional ethics committee (IRB #5891).

NAION group

The group of patients suffering from NAION included 57 patients aged 18 years or over with the following inclusion criteria: (1) a history of sudden (less than 14 days between the first symptoms and the examination) painless loss of vision, (2) optic disc edema at the time of visual loss, (3) visual field defects consistent with optic-disc pathology, (4) absence of another neurologic or ocular disorder that may be responsible for optic disc edema and visual impairment, and (5) exclusion of arteritic anterior ischemic optic neuropathy (AAION, Horton disease). The resolution phase of AION was defined by the disappearance of the optic nerve edema.[8] Exclusion criteria were patients with ametropia > 5 diopters (spherical equivalent) or with any other ocular disease.

Diurnal hypertension was defined as daytime systolic blood pressure of at least 135 mm Hg, or daytime diastolic blood pressure of at least 85 mm Hg.

Control group

57 controls were included in the same clinic and matched to NAION patients for sex, age (10-year interval), systemic hypertension, diabetes and OSA. Exclusion criteria were patients with ametropia > 5 diopters (spherical equivalent) or with any ocular disease. Medi-

cal history, particularly cardiovascular risk factors, high-blood pressure, diabetes, dyslipidemia and smoking, was collected on the basis of self-declaration. Epworth and NoSAS scores [9,10] were obtained for all controls without previously known OSA syndrome, in order to exclude control subjects with an Epworth score > 8 and / or a NoSAS score> 8.

Clinical data and imaging protocol

All patients and control subjects underwent a complete ocular examination including visual acuity (VA) and measurement of intraocular pressure (IOP, non-contact tonometry, TONOREF II, Nidek™, Gamagori, Japan). Anterior segment slit lamp examination and fundus examination were performed to rule out ocular diseases.

45-degree funduscopic color photographs were acquired (one per eye), centered on the optic nerve and the macula, using a non-mydriatic camera, specifically: TopCon TRC 50 IX (Topcon, Tokyo, Japan) for 15 funduscopic color images; TopCon TRC NW6S (Topcon, Tokyo, Japan) for 36 funduscopic color images; Canon CR-2 (Canon, Tokyo, Japan) for 56 funduscopic color images**.**

Following a well-established procedure,[11] the pixel-to-mm conversion factor was obtained by dividing the average vertical optic disc diameter (ODD) over all images, acquired with the same camera at the same resolution by the assumed average disc diameter in microns (1850 µm), The resulting conversion factor (pixels / microns) was 5.43 for the 3008x2000 TOPCON TRC 50IX images, 7.31 for the 2240x1488 TOPCON TRC 50IX images, 6.43 for the 3008x2000 TOPCON TRC NW6S, 5.65 for the 5184x3456 Canon CR2 images and 8.03 for the 2592x3456 Canon CR2 images.

Image analysis

VAMPIRE measures semi-automatically morphological parameters of the retinal vessels in fundus images. Details and validation have been reported elsewhere [5,7,14,21]. Measurements (Table 3, supplemental material) include CRAE and CRVE, summarizing the widths of the six largest arteries and venules respectively (from the revised formulas of Knudtson);[12] AVR = CRAE/CRVE; the FD, a measure of the geometric complexity of the vessel network in zone C, is computed as the generalized dimensions D0, D1 and D2, which correspond, respectively, to the capacity (or box-counting) dimension, information (or Shannon) dimension, and correlation dimension of the vascular network;[13,30] and arterial and venular vascular tortuosity, averaged respectively over the six largest arteries and venules.[14]

Briefly, VAMPIRE starts by locating the optic disc contour and the macula center, enabling the definition of the usual retinal coordinates (x axis through OD and macula centers, origin in the OD center) and circular zones around the OD (Figure 1): zone A (between OD center and 0.5 ODD), zone B (between 0.5 and 1 ODD), and zone C (between 0.5 and 2 ODD). Efficient manual correction can be performed when the automatic location of the optic disc or fovea are found to be incorrect. Vessels are subsequently detected and labeled as arterioles or venules automatically, and again corrected manually. AVR, CRAE and CRVE are computed in zone B, FD and vascular tortuosity in zone C. Raw measurements of CRAE and CRVE are in pixels.

Statistical analysis

Categorical data are reported as numbers and percentages and continuous variables as mean and standard deviation or median and 25^{th}-75^{th} percentiles, where appropriate. For univariate analysis, we compared retinal vascular parameters between NAION cases and controls using the Wilcoxon matched-pairs signed-ranks test.

For subgroup analysis, we examined whether the differences in retinal vascular parameters between NAION cases and controls varied with the NAION stage. For this purpose, we performed multivariate linear regression with retinal vascular parameters as dependent variable (Y_i), as follows:

$$Y_i = \beta_0 + \beta_1 NAION_i + \beta_2 Stage_i + \delta(NAION_i \times Stage_i) + e_i$$

where, for the *i*-th subject, *NAION* is a binary variable indicating whether the subject is a NAION case (value 1) or control (value 0), *Stage* is a binary variable coding whether the NAION case is enrolled during the acute (value 1) or resolutive (value 0) stage, δ is 1 when the patient is NAION (otherwise 0), and *e* is the idiosyncratic error term. The ***β*** regression coefficients are the estimated effects of a unit change in each independent variable on the retinal vascular parameter. We tested the first-order interaction involving the *NAION* and *Stage* terms (i.e., *γ*) for statistical significance. To account for case-control matching, we used Generalized Estimated Equations.
Two-sided p-values <0.05 were considered statistically significant. All analyses were performed using Stata Special Edition version 14.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Study sample

Eighty-two patients were screened and 25 patients were excluded from the study sample due to the diagnosis of AAION (n=7), low image resolution (resolution <1080 pixels, n=4), rejection by the software (n= 14, due to blur or i inadequate centering). 57 patients were included in the analysis after exclusion, of which 35 had acute NAION and 22 were evaluated at the time of resolution of the optic nerve edema. The mean delay of resolving papillary edema was 45 days. Fifty-seven patients were included in the control group. Baseline characteristics for NAION cases and controls are summarized in Table 1.

NAION vs. matched control subjects

Compared with controls, the median of AVR, arteriolar and venular tortuosity values of NAION cases were consistently lower, both in the full analytical sample and within each subgroup, with no evidence of significant interaction according to NAION stage (Table 2).

In contrast, the observed differences in median CRAE and CRVE values between NAION cases and controls varied with the AION stage (p for interaction = .001 and .03, respectively, Table 2). NAION patients had higher median CRAE and CRVE values than controls for acute NAION, but not so at the resolution stage. Yet, the findings from the subgroup analysis for CRAE should be interpreted with caution as the difference between NAION cases and controls in the full analytical sample was not significant (*P* = .41, Table 2).

Substantial variability was observed in median D0a values among controls, while median D0a values (i.e., 1.55, Table 2) were similar for NAION cases across study subgroups defined by NAION stage. This was evidenced by the significant first-order interaction in subgroup analysis for D0a. This latter observation might reflect random variability or confounding by an extraneous factor in D0a values among controls.

DISCUSSION

This study suggests that eyes with NAION differ from controls in having higher CRVE and lower AVR and vascular tortuosity. Acute NAION eyes showed increased CRAE and CRVE, whereas eyes at the resolution stage exhibited reduced CRAE, similar CRVE and increased D0a in comparison with controls.

To our best knowledge, this is the first study exploring the retinal vessel phenotype from fundus images in NAION. Comorbidities associated with NAION, such as age, systemic hypertension and diabetes,[15] have been linked with changes in CRAE, CRVE or AVR. At this time, retinal vascular changes in OSA are unknown; to consider potential biases, we compared NAION patients with control subjects matched for sex, age, systemic hyperten-

sion, diabetes as well as OSA. We can therefore assume that differences between NAION and control groups are related to the occurrence of NAION itself.

The first important result is the increase in CRVE at the acute stage of NAION. This change is no longer detected at resolution stage, suggesting that dilation of veins is present at the time of initial damage. The same trend was noted for CRAE. These data are consistent with the hypothesis of venous congestion within the anterior optic nerve suggested by Levin et al. [16] and with the concept of compartment syndrome.[17] Further research and larger cohorts are needed to understand these retinal vascular changes, especially if they are related to vascular changes within the optic nerve or if they only reflect temporary changes within the retina without simultaneous vascular changes within the optic nerve. Magnetic resonance imaging angiography could be explored to study ophthalmic artery and orbital veins, vessels widths, velocity, insufficiency and thrombosis.

The second relevant result is that tortuosity was significantly lower in the NAION group than in the control group, whatever the stage of NAION. Tortuosity (a dimensionless retinal vessel trait) has been previously associated with axial length and spherical equivalent,[18] which we took into account when matching patients and controls. Reduced arterial tortuosity has been associated with age, elevated blood pressure and higher body mass index, all factors controlled by our matching strategy.[18,19] Therefore, lower tortuosity seems to be a constant vascular parameter associated with NAION, both at the early and resolution stage of the disease. This vascular trait should be further tested as a risk factor for N-AION. Retinal tortuosity should also be studied further as a vascular marker of changes in the vascular network of the optic nerve.

We found that fractal dimension (D0a) did not change significantly at different disease stages. The FD captures the complexity of the retinal vascular tree as a geometric pattern, including the degree of branching complexity.[20] We used a multifractal approach which calculates multiple fractal dimensions from ~1,000 randomly chosen starting points on the vessel tree. As this measure considers the retinal vascular system as a whole and at different scales, it is accepted as a quantitative measure of the complexity of the overall vascular architecture.[21] Our results show a substantial variability in median D0a values among controls, while median D0a values (i.e., 1.55, Table 2) were similar for NAION cases across study subgroups defined by NAION stage. Larger cohorts are necessary to confirm the variability of the FD.

Finally, this study does not allow us to conclude whether changes of retinal vascular parameters precede the occurrence of NAION, nor whether it is an associated phenomenon or a consequence of vascular abnormalities in the optic nerve. A longitudinal study is necessary to understand the evolution of the retinal vascular parameters. Once the optic nerve edema resolves, some retinal changes seem to disappear, as reported in our transversal study. Our results could, however, suggest that these retinal abnormalities are associated with the evolution of the disease, since control and NAION groups shares the same risk factors susceptible to change retinal vascular parameters.

We do acknowledge several limitations to our study. First, the size of our cohort of NAION patients is modest. Second, the results of case-control study design, even though largely used in the literature, may be sensitive to confounding unmeasured factors. Hence some of the associations observed in subgroup analysis might be spurious or explained by random variability. For this reason, the findings from subgroup analysis for CRAE and D0a parameters especially should be interpreted with caution. A longitudinal study is also needed in order to better understand the time changes in vascular parameters between the acute and resolution stage. Third, CRAE and CRVE cannot identify differences in diameters between peripapillary and distal vessels.[4] Direct measurements of vessel widths along vessel segments would be preferable, and can be obtained from VAMPIRE software. Fourth, the potential variability of the magnification error due to different cameras has to be taken into account; here, its effect was limited by the use of a pixel-micron conversion factor adjusted to the average optic disc size and the fundus camera. Finally, the stability and repeatability of FD measures is the object of ongoing analysis.[31]

In conclusion, to our best knowledge, this is the first study of the retinal vessel phenotype in patients with non-arteritic anterior ischemic optic neuropathy. This case-control study showed that NAION patients exhibited more frequently arteriolar and venular dilations. Retinal vascular changes differ from the acute to the resolution stage, which suggests that some vascular regulation takes place in the latter. Lower tortuosity and higher FD found in NAION patients should be further studied since they could represent a vascular surrogate within the retina associated with the occurrence of NAION.

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Association for Research and Teaching in Ophthalmology (ARFO, Grenoble, France). The sponsor of the funding organization had no role in the design or conduct of this research.

REFERENCES

1. Hayreh SS. Ischemic optic neuropathy. *Prog Retin Eye Res*. 2009;28(1):34‑62.

2. Hayreh SS, Joos KM, Podhajsky PA, Long CR. Systemic diseases associated with nonarteritic anterior ischemic optic neuropathy. *Am J Ophthalmol*. 1994;118(6):766‑80.

3. Hayreh SS, Zimmerman MB, Podhajsky P, Alward WL. Nocturnal arterial hypotension and its role in optic nerve head and ocular ischemic disorders. *Am J Ophthalmol*. 1994;117(5):603‑24.

4. Rader J, Feuer WJ, Anderson DR. Peripapillary vasoconstriction in the glaucomas and the anterior ischemic optic neuropathies. *Am J Ophthalmol.* 1994;117(1):72‑80.

5. MacGillivray TJ, Cameron JR, Zhang Q, et al. Suitability of UK Biobank Retinal Images for Automatic Analysis of Morphometric Properties of the Vasculature. *PloS One*. 2015;10(5):e0127914.

6. McGrory S, Taylor AM, Kirin M, et al. Retinal microvascular network geometry and cognitive abilities in community-dwelling older people: The Lothian Birth Cohort 1936 study. *Br J Ophthalmol*. 2017;101(7):993‑8.

7. Perez-Rovira A, MacGillivray T, Trucco E, et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. *Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf*. 2011:3391‑4.

8. Contreras I, Noval S, Rebolleda G, Muñoz-Negrete FJ. Follow-up of nonarteritic anterior ischemic optic neuropathy with optical coherence tomography. *Ophthalmology*. 2007;114(12):2338‑44.

9. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. 1991;14(6):540‑5.

10. Marti-Soler H, Hirotsu C, Marques-Vidal P, et al. The NoSAS score for screening of sleep-disordered breathing: a derivation and validation study. *Lancet Respir Med.* 2016;4(9):742‑8.

11. Creuzot-Garcher C, Binquet C, Daniel S, et al. The Montrachet Study: study de-

sign, methodology and analysis of visual acuity and refractive errors in an elderly population. *Acta Ophthalmol (Copenh)*. 2016;94(2):e90-97.

12. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res.* 2003;27(3):143‑9.

13. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. *Neurology*. 2010;74(14):1102‑7.

14. Lisowska A, Annunziata R, Loh GK, Karl D, Trucco E. An experimental assessment of five indices of retinal vessel tortuosity with the RET-TORT public dataset. *Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf.* 2014:5414‑7.

15. Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. *Ophtalmol Int J Ophthalmol Z Augenheilkd.* 2013;229(3):125‑36.

16. Levin LA, Danesh-Meyer HV. Hypothesis: a venous etiology for nonarteritic anterior ischemic optic neuropathy. *Arch Ophthalmol Chic Ill 1960.* 2008;126(11):1582‑5.

17. Tesser RA, Niendorf ER, Levin LA. The morphology of an infarct in nonarteritic anterior ischemic optic neuropathy. *Ophthalmology*. 2003;110(10):2031‑5.

18. Lim LS, Cheung CY, Lin X, Mitchell P, Wong TY, Mei-Saw S. Influence of refractive error and axial length on retinal vessel geometric characteristics. *Invest Ophthalmol Vis Sci.* 2011;52(2):669‑78.

19. Taarnhøj NC, Munch IC, Sander B, et al. Straight versus tortuous retinal arteries in relation to blood pressure and genetics. *Br J Ophthalmol.* 2008;92(8):1055‑60.

20. Masters BR. Fractal analysis of the vascular tree in the human retina. *Annu Rev Biomed Eng.* 2004;6:427‑52.

21. Macgillivray TJ, Patton N, Doubal FN, Graham C, Wardlaw JM. Fractal analysis of the retinal vascular network in fundus images. *Conf Proc Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf.* 2007:6456‑9.

22. Grauslund J, Green A, Kawasaki R, Hodgson L, Sjølie AK, Wong TY. Retinal vascular fractals and microvascular and macrovascular complications in type 1 diabetes. *Ophthalmology*. 2010;117(7):1400‑5.

23. Cheung CY, Thomas GN, Tay W, et al. Retinal vascular fractal dimension and its relationship with cardiovascular and ocular risk factors. *Am J Ophthalmol.* 2012;154(4):663-674.e1.

24. Azemin MZC, Kumar DK, Wong TY, et al. Age-related rarefaction in the fractal dimension of retinal vessel. *Neurobiol Aging.* 2012;33(1):194.e1-4.

25. Liew G, Wang JJ, Cheung N, et al. The retinal vasculature as a fractal: methodology, reliability, and relationship to blood pressure. *Ophthalmology*. 2008;115(11):1951‑6.

26. Sng CCA, Wong WL, Cheung CY, Lee J, Tai ES, Wong TY. Retinal vascular fractal and blood pressure in a multiethnic population. *J Hypertens*. 2013;31(10):2036‑42.

27. Kurniawan ED, Cheung N, Cheung CY, Tay WT, Saw SM, Wong TY. Elevated blood pressure is associated with rarefaction of the retinal vasculature in children. *Invest Ophthalmol Vis Sci*. 2012;53(1):470‑4.

28. Kawasaki R, Che Azemin MZ, Kumar DK, et al. Fractal dimension of the retinal vasculature and risk of stroke: a nested case-control study. *Neurology*. 2011;76(20):1766‑7.

29. McGrory S, Cameron JR, Pellegrini E, et al. The application of retinal fundus camera imaging in dementia: A systematic review. *Alzheimers Dement Amst Neth*. 2017;6:91‑107.

30. Stošic T and Stošic BD. Multifractal analysis of human retinal vessels. *IEEE Trans Medical Imaging.* 2006;25:1101-1107.

31. Huang F, Jiang J, Bekkers EJ, Dashtbozorg B, Ter Haar Romeny BT. Stability Analysis of the Fractal Dimension of the Retinal Vasculature. *Proc. MICCAI Int. Workshop on Ophthalmic Medical Image Analysis*, 2015.

FIGURE

Figure 1: Retinal Zones and segmentation using Vampire
1A: Example of fundus image of one eye with NAION, and segmentation of retinal vein or artery vessels. Zones A, B and C are used in the standard reference system based on center and radius of the optic nerve disc (OD), and on the location of the fovea. The system divides the image in concentric regions around the OD center. The zones are defined as zone A (between OD center and 0.5 ODD), zone B (between 0.5 and 1 ODD), and zone C (between 0.5 and 2 ODD). Periphery is defined as the area outside Zone C.
1B: example of fractal dimension analysis of the same ocular fundus.

TABLES:

Table 1: Demographics of patients and control subjects
Patients were matched for sex, systemic hypertension, OSA and diabetes.
OSA: obstructive sleep apnea syndrome.

Table 2: Difference in retinal vascular parameters between NAION cases and controls stratified according AION stage.
Values for FD were missing for 11 NAION and 13 control patients.
AVR: Arteriole to venule ratio; CRAE: Central retinal arterial equivalent; CRVE: Central retinal venular equivalent; OD: optic disc, ZoneCATort: Average tortuosity of the largest arterioles in zone C; ZoneCVTort: Average tortuosity of the largest venules in zone C; FD: Fractal dimension; Num1stBa: number of first branching points in arterial vessel trees detected in zone C ; Num1stBv: number of first branching points in venular vessel trees detected in zone C.

Table 3 (supplemental material): Description of main vascular parameters measured using VAMPIRE software. Zone B and C are illustrated in Figure 1.

Table 1

Characteristics*	Control (n=57)	NAION (N=57)	Acute stage of NAION (n=35)	Resolution stage of NAION (n=22)	p value (NAION vs control group)
Female gender, *n (%)*	25 (44)	25 (44)	13 (37)	12 (55)	*
Eye *n (%)*					0.77
Right	34	32	20	12	
Left	23	25	15	10	
Age, mean (SD)	71 (8)	70 (8)	69 (8)	73 (7)	*
Systemic hyperten-sion	31 (54%)	31 (54%)	18 (51%)	13 (59%)	*
OSA	24 (42%)	24 (42%)	15 (43%)	9 (41%)	*
Diabetes	12 (21%)	12 (21%)	9 (26%)	3 (14%)	*

Table 2

Table 2. Difference in retinal vascular parameters between NAION cases and controls stratified according AION stage.

Retinal vascular parameter	Stage	NAION cases (1)		Controls (2)		Difference (1 – 2)		***P***
		n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	
CRAE, *μm*	All	57	156 (147;177)	57	160 (150;168)	57	6 (-19;19)	.41
	Acute	35	172 (149;182)	35	161 (155;170)	35	11 (-19;37)	
	Resolution	22	151 (147;157)	22	152 (143;167)	22	-8 (-17;9)	.03*
CRVE, *μm*	All	57	232 (206;256)	57	210 (200;224)	57	16 (-6;47)	<.001
	Acute	35	241 (216;270)	35	210 (200;227)	35	28 (6;65)	
	Resolution	22	209 (196;236)	22	212 (197;221)	22	4 (-13;17)	.001*
AVR	All	57	0.71 (0.66;0.76)	57	0.76 (0.71;0.79)	57	-0.03 (-0.14;0.04)	.01
	Acute	35	0.70 (0.66;0.76)	35	0.78 (0.71;0.81)	35	-0.06 (-0.14;0.05)	
	Resolution	22	0.72 (0.68;0.77)	22	0.74 (0.68;0.78)	22	0.00 (-0.05;0.03)	.23*
ZoneCATort, $\times 10^{-5}$	All	57	6.7 (2.5;13.3)	57	27.4 (18.7;53.2)	57	-20.5 (-50.9;-9.3)	<.001
	Acute	35	6.8 (2.6;17.2)	35	30.2 (17.1;79.0)	35	-22.2 (-75.2;-7.2)	
	Resolution	22	5.5 (2.3;11.7)	22	24.5 (18.7;43.0)	22	-19.6 (-36.0;-9.3)	.14*
ZoneCV Tort, $\times 10^{-5}$	All	57	7.2 (3.2;13.1)	57	19.9 (10.2;45.8)	57	-15.2 (-37.2;-1.4)	<.001
	Acute	35	8.3 (3.6;17.3)	35	21.8 (10.3;41.8)	35	-16.7 (-29.6;-0.3)	
	Resolution	22	5.4 (2.8;10.0)	22	16.0 (8.8;54.0)	35	-11.1 (-43.5;-3.2)	.91*
Num1stBA	All	57	2 (1;3)	57	2 (1;3)	57	0 (-1;1)	.64
	Acute	35	2 (1;2)	35	2 (1;3)	35	-1 (-2;1)	
	Resolution	22	2 (1;3)	22	2 (1;2)	22	1 (-1;1)	.08*

(Continued)

Table 2 (Continued)

Retinal vascular parameter	Stage	NAION cases (1)		Controls (2)		Difference (1 – 2)		*P*
		n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	
Num1stBV	All	57	2 (1;3)	57	2 (2;3)	57	0 (-1;1)	.20
	Acute	35	2 (1;3)	35	2 (2;3)	35	0 (-1;1)	
	Resolution	22	2 (1;3)	22	2 (1;3)	22	-0.5 (-2;1)	.32*
D0a	All	46	1.55 (1.51;1.59)	44	1.52 (1.48;1.57)	34	0.04 (-0.02;0.11)	.02
	Acute	29	1.55 (1.50;1.59)	25	1.54 (1.48;1.58)	20	0.01 (-0.05;0.08)	
	Resolution	17	1.55 (1.54;1.59)	19	1.50 (1.46;1.56)	14	0.07 (0.00;0.11)	.04*
D1a	All	46	1.54 (1.50;1.58)	44	1.53 (1.49;1.57)	34	0.01 (-0.02;0.06)	.33
	Acute	29	1.54 (1.49;1.58)	25	1.54 (1.49;1.57)	20	-0.01 (-0.09;0.06)	
	Resolution	17	1.54 (1.53;1.58)	19	1.52 (1.47;1.56)	14	0.03 (-0.01;0.07)	.19*
D2a	All	46	1.53 (1.50;1.58)	44	1.51 (1.48;1.56)	34	0.01 (-0.04;0.06)	.40
	Acute	29	1.53 (1.48;1.57)	25	1.52 (1.48;1.56)	20	-0.01 (-0.07;0.05)	
	Resolution	17	1.53 (1.51;1.58)	19	1.51 (1.46;1.55)	14	0.02 (-0.01;0.07)	.34*
D0v	All	46	1.54 (1.49;1.57)	44	1.51 (1.47;1.55)	34	0.03 (-0.02;0.08)	.06
	Acute	29	1.53 (1.49;1.57)	25	1.52 (1.47;1.56)	20	0.01 (-0.03;0.07)	
	Resolution	17	1.54 (1.51;1.57)	19	1.50 (1.45;1.54)	14	0.05 (-0.01;0.09)	.60*
D1v	All	46	1.53 (1.49;1.56)	44	1.51 (1.48;1.56)	34	0.01 (-0.03;0.07)	.41
	Acute	29	1.53 (1.48;1.56)	25	1.52 (1.48;1.56)	20	-0.01 (-0.04;0.06)	
	Resolution	17	1.53 (1.50;1.56)	19	1.51 (1.46;1.55)	14	0.03 (-0.02;0.07)	.59*
D2v	All	46	1.52 (1.48;1.56)	44	1.51 (1.47;1.55)	34	0.01 (-0.03;0.07)	.42
	Acute	29	1.52 (1.47;1.56)	25	1.52 (1.47;1.56)	20	-0.01 (-0.04;0.06)	
	Resolution	17	1.53 (1.49;1.56)	19	1.50 (1.45;1.54)	14	0.03 (-0.02;0.07)	.57*

Abbreviations: AVR, arteriole-to-venule ratio; CRAE, central retinal arterial equivalent; CRVE, central retinal venular equivalent; IQR, interquartile range (i.e., 25-75th percentiles); NAION, non-arteritic ischemic optic neuropathy; Num1stBa, number of first branching points in arterial vessel trees detected in

zone C; Num1stBV, number of first branching points in venular vessel trees detected in zone C; ZoneCATort, average tortuosity of the largest arteriole in zone C; ZoneCV Tort, average tortuosity of the largest venule in zone C.

* *P* for first-order interaction term involving NAION status and stage.

Figure 1A

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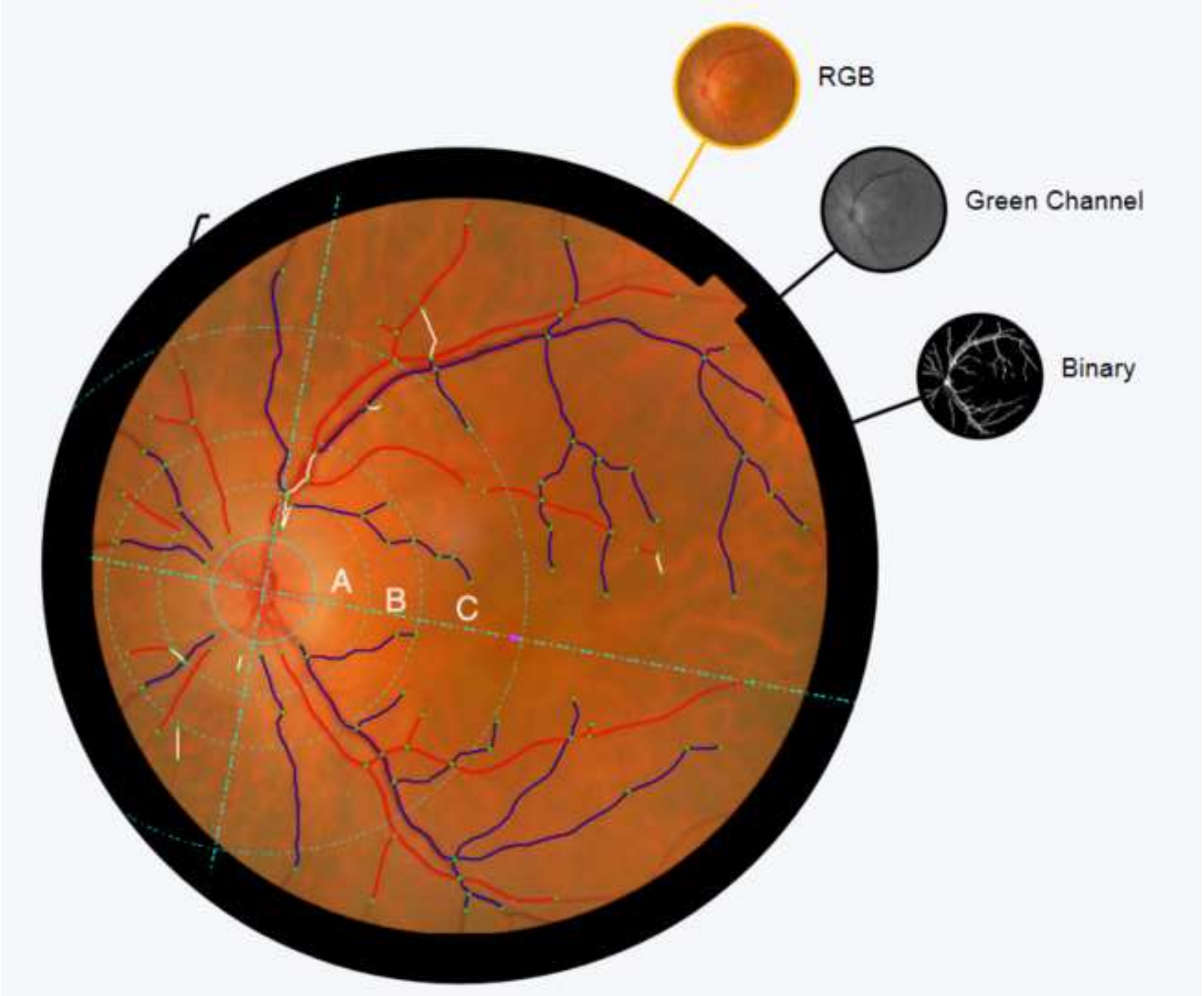

Figure 1B

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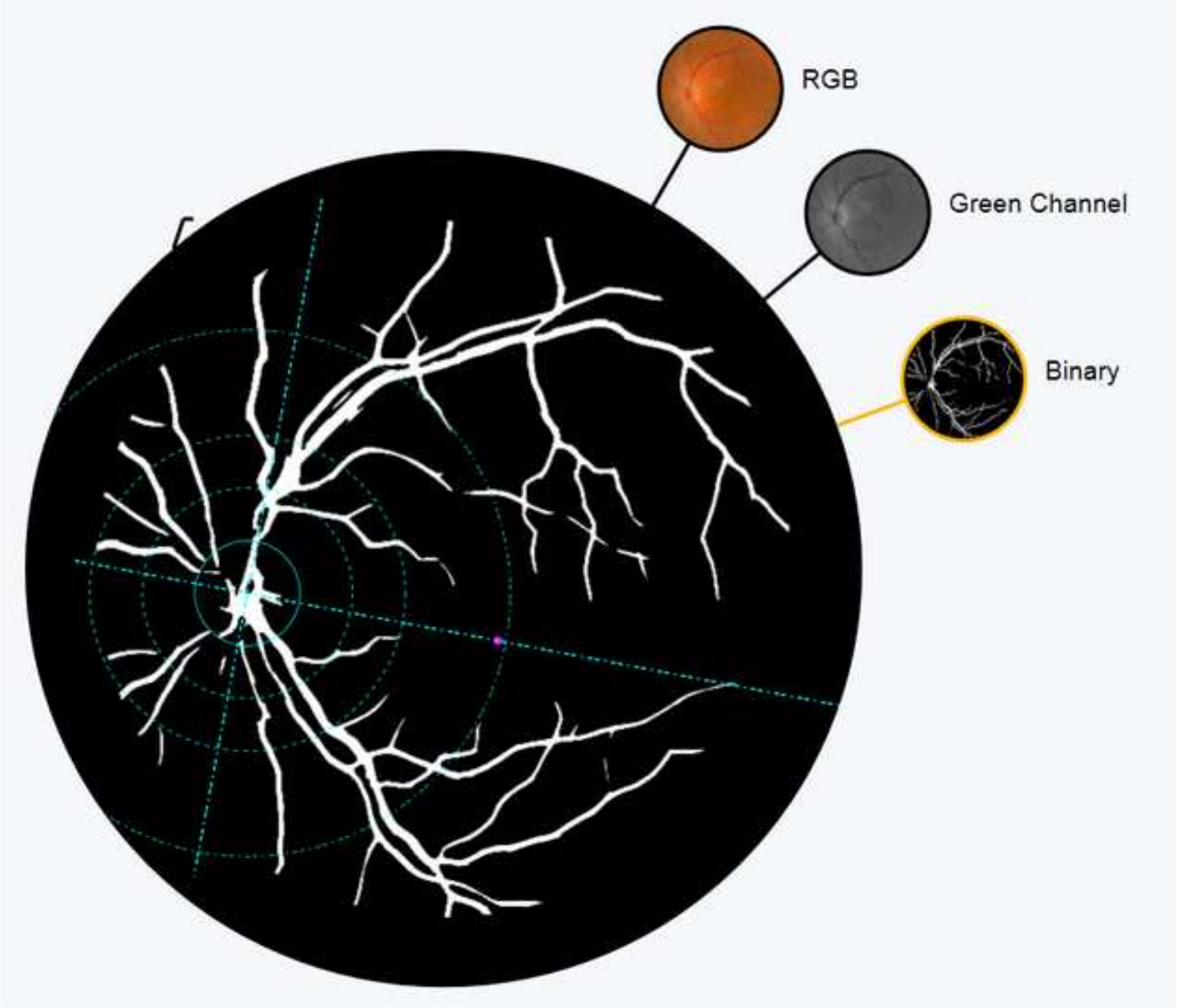

***Table of Contents Statement**

This retrospective case series with matched controls showed that non-arteritic anterior ischemic optic neuropathy patients exhibited arteriolar and venular dilations at the acute stage more frequently than controls. Retinal vascular changes differed between the acute and the resolution stage, suggesting that some vascular regulation takes place in the latter. In the resolution stage, arterial and venular width values normalized, whereas the fractal dimension increased.

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CRAE	A summative measure of arteriolar width in zone B, based on the widths of the six largest arteries (Figure 1, vessels in red), and a function of coefficients calculated from the width of arteries at bifurcations.
CRVE	A summative measure of venular width in zone B, based on the widths of the six largest venules (Figure 1, vessels in blue), and a function of coefficients calculated from the width of venules at bifurcations.
Fractal dimension	Retinal vasculature is a complex branching structure. Its complexity is not easily captured by conventional geometric measurements. The fractal dimension (FD) was introduced to quantify the complexity of self-similar structures, i.e. geometric patterns repeating themselves over multiple dimensions, or spatial scales. The dimensionless FD has become an accepted measure of the complexity of the retinal vascular network (Figure 1). See the main text for pointers to the main types of FD and algorithms to compute them.
Tortuosity	Tortuosity measures how twisted vessels are. The simple arc-to-chord ratio fails to take into account multiple points of inflection along a vessel segment. The VAMPIRE software adopts the best performing algorithm identified in the experimental comparison by Lisowska et al.[(14)] Tortuosity is averaged over the six largest arteries and venules in zone C.

TABLE 3: Description of main vascular parameters measured using VAMPIRE software. Zone B and C are illustrated in Figure 1.